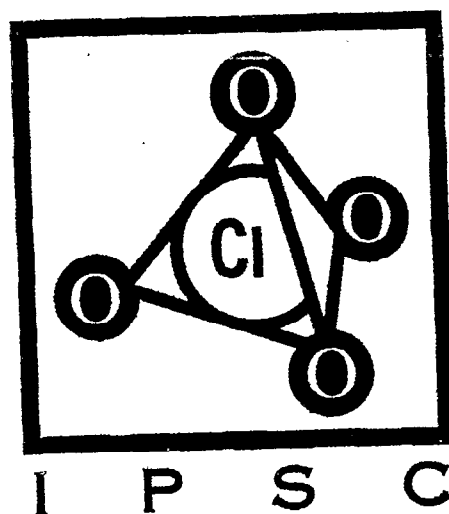


# Perchlorate Ecological Risk Studies



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Subcommittee

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# Perchlorate Ecological Risk Studies

## Introduction

There is currently a consensus that ecological risk assessment follows the same fundamental paradigm as human health risk assessment (U.S. EPA, 1992). The paradigm is a three phase structure that includes problem formulation, analysis, and risk characterization. In addition there is a fundamental premise that in order for risk to exist there must be both exposure to a "stressor" and the stressor must have the inherent ability to cause adverse effects to receptor populations at ecologically significant concentrations. Relative to the ecological risk from perchlorate, both the exposure to environmental receptors and the inherent toxicity of perchlorate have numerous and substantial information gaps.

With respect to the potential for perchlorate to cause ecological risk, there is limited information, specifically, on the "inherent" ability of perchlorate to cause adverse ecological responses. This document discusses the information available on perchlorate toxicity relative to ecological risk assessment, followed by a discussion of the rationale for the selection of the toxicity tests, which were conducted as part of this effort. Results of the toxicity tests are then presented, followed by an ecological risk evaluation, which includes an evaluation of exposure pathways and recommendations for further studies.

## Background

Perchlorate anion ( $\text{ClO}_4^-$ ) exists as a contaminant in ground water and surface waters from the solid salts of ammonium, potassium, or sodium perchlorate. Ammonium perchlorate is manufactured for use as an oxidizer component in solid propellant for rockets, missiles, and fireworks. Because of its relatively short shelf life, it must be periodically washed out of the country's missile and rocket inventory and replaced with a fresh supply. Large volumes of the compound have been disposed of in California, Utah, and, likely, other sites, since the 1950's. Potassium perchlorate had, until recently, been used therapeutically to treat hyperthyroidism resulting from an autoimmune condition known as Grave's disease (Lamm, 1998). Potassium perchlorate is still used diagnostically to test thyroid hormone (TSH, T3 and T4) production in some clinical settings. It is believed that the mechanism of action on the thyroid functioning is the competitive inhibition of the uptake of iodine in the thyroid gland.

Thyroid hormone deficiencies can affect normal metabolism, growth and development. The limited database on the toxicology of perchlorate confirms its potential to disrupt thyroid hormone production in mammalian test species. While virtually all vertebrates (fish, amphibians, reptiles, birds and mammals) have thyroid glands, no robust data on perchlorate exists to evaluate these organisms. There are no data to evaluate uptake of perchlorate into vegetation, either native plants or agricultural species. In addition, there are no robust data available to conclude whether or not other mechanisms of toxicity (other than thyroid effects) exist in animals or plants.

Perchlorate salts are quite soluble in water. The resultant anion ( $\text{ClO}_4^-$ ) is exceedingly mobile in aqueous systems and can persist for many decades under typical groundwater and surface water conditions due to kinetic barriers to its reactivity with other available constituents.

## Literature Review

Searches of available databases have revealed minimal information on the potential ecological effects of ammonium perchlorate or any of the other perchlorate salts. Essentially no data exists for perchlorate effects on most soil, sediment, or aquatic receptors including aquatic vertebrates, aquatic invertebrates (water column or sediment dwelling), bacteria or plants. Limited data do suggest that the thyroid-hormone functioning of the South African clawed frog, *Xenopus laevis*, can be affected by exposure to perchlorate in the range of 50 -100 parts per million (ppm) (Coleman et al, 1968) . In recent studies, 1000 ppm has been shown to completely block the metamorphosis of tadpoles (Kinney et al, 1996). A concentration of 340 ppm potassium perchlorate was used to block metamorphosis and affect thyroid functioning in the South American toad *Bufo arenarum*. Effects on development and growth have also been demonstrated in the sea lamprey at 100 ppm (Youson et al, 1995) and the freshwater hydra at 350 ppm (Confer et al, 1996). Mortality was observed in cold water trout between 6000 and 7000 ppm (Kuroda, 1974) and *Daphnia magna* were effected at 670 ppm (Bringmann and Kuhn, 1977). Effects on seed germination and growth of agricultural plants were reported at 10 ppm (Naqvi and Latif, 1976).

The conclusions from a 1996 developmental toxicity test performed by the U.S. Air Force stated that for *Hydra* the exposure concentration which could affect development was effectively the same as the exposure concentration which caused maternal toxicity--that is, ammonium perchlorate was not developmentally toxic beyond its toxicity to fully developed *Hydra* (Confer et al, 1996). A confounding factor with this study is that the tests used ammonium perchlorate. It is possible that the adverse effects seen in these tests were influenced by ammonium toxicity in addition to any perchlorate toxicity.

Studies conducted for NASA suggest that ammonium perchlorate may adversely affect plants species (Naqvi and Latif, 1975). However, there is, again, the confounding factor of the ammonium ion in the tests which may account for the observed effects. In addition, the method of exposure for the study was based on an application rate per unit area of soil, not concentration based. This limits the ability to interpret this study, as exposure relationships needed for ecological risk evaluation cannot be determined.

In a study on the cause of leaf malformation in soybeans grown in Chile, it was determined that the presence of potassium perchlorate in a Chilean fertilizer (containing between 0.12 and 0.26% potassium perchlorate) was responsible for the observed effects (Tollenaar and Martin, 1972). However, a concentration-based exposure rate could not be determined from the study design.

Recently a study was completed on the effect of ammonium perchlorate using the FETAX (Frog

Embryo Teratogenesis Assay: *Xenopus*) test (Dumont and Bantle, 1998). The conclusion of this assay was that the LC<sub>50</sub> for ammonium perchlorate was 496 ppm, the average effective concentration (EC<sub>50</sub>) for malformations was found to be about 396 ppm. It was concluded that ammonium perchlorate is not a significant teratogenic threat, but is an embryonic toxicant.

### Rationale for Selected Toxicity Tests

Several factors form the foundation for the selection of the current screening level ecological testing. These factors follow from the review of the existing data base on potential ecological effects from perchlorate. Previous tests used ammonium perchlorate as the test compound and ammonium is known to be a strong toxicant, thereby confounding the test result relative to perchlorate toxicity. Second, there are data which suggest that perchlorate may have a mechanism of toxicity which is not related to thyroid functioning. Other mechanisms of toxicity can not be discounted. Third, the data base for perchlorate effects on ecological receptors is generally weak. Fourth, because of the use of perchlorate contaminated Colorado River water for irrigation, the use of an agricultural test species may provide useful information outside of the ecological risk assessment.

Potential tests were evaluated for their availability, rapid turnaround, interpretation power, and representativeness of a spectrum of organism classes. Through the current approach an evaluation is being made as to whether or not effort should be expended in the evaluation of other effects of perchlorate exposure at ecologically significant levels.

The test species selected were *Daphnia magna* and *Ceriodaphnia dubia* (free swimming freshwater aquatic invertebrates commonly called water fleas), *Pimephales promelas* (fathead minnow), *Eisenia foetida* (the redworm), and *Lactuca sativa* (a leaf lettuce). These five organisms encompass two invertebrate species (aquatic and terrestrial), an aquatic vertebrate, and a plant.

Toxicity tests were performed in accordance USEPA and ASTM guidance following the contractor's standard toxicity testing protocols. A graded concentration series of five ClO<sub>4</sub> concentrations, dilution water/dilution media controls, and sodium controls were used in each assay. The laboratory was tasked to conduct range finding tests (based upon existing data for a upper end starting concentration) and definitive tests to determine an LC<sub>50</sub>. These tests are standard tests which have known interpretive strengths and weaknesses.

The *D.magna* test is the 48-hour test (ASTM E 729-88a. The LC<sub>50</sub> was calculated based on number of surviving organisms per concentration using Spearman Karber, Trimmed Spearman Karber, or probit analysis.

*Ceriodaphnia dubia* chronic toxicity testing followed ASTM E729-88a testing protocol. A 48-hour LC<sub>50</sub> was calculated from each *C. dubia* chronic toxicity test. Following USEPA guidance, survival and reproduction data was analyzed to determine if test concentrations were significantly

different from controls. Test endpoints were expressed as the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Level (LOEC), the Chronic Value (ChV). Additionally, a 25 percent Inhibition Concentration (IC<sub>50</sub>) was calculated for each test. The *P. promelas* test is the 96-hour test (ASTME 729-88a).

The *E. foetida* test is a 14-day test with a mortality endpoint. This test followed the procedures of the ASTM Standard Guide E 1676-95 entitled "Conducting a Laboratory soil Toxicity Test with Lumbricid Earthworm *Eisenia foetida*". The object was to generate a soil LC<sub>50</sub> based on 7-day and 14-day survival data.

The *L. sativa* (lettuce) test is a 28-day growth test following the procedures of ASTM Standard Guide E 1598-94 "Conducting Early Seedling Growth Test". The objective of the test is to determine a NOEC, LOEC, ChV, and an IC<sub>50</sub> for lettuce. The percent germination, percent survival, and mean dry weight data were statistically analyzed to determine which test concentrations were significantly different from control (P=0.05).

### **Ecological Risk Evaluation of Perchlorate**

This ecological risk assessment (ERA) follows the Superfund paradigm for conducting ecological risk assessments (USEPA, 1997). This effort, to evaluate the potential risk of ammonium perchlorate, consists of an evaluation of the toxicity of ammonium perchlorate and exposure analysis, followed by a risk characterization. Assessment endpoints for this effort include aquatic food resources for fish species, plant productivity, and soil structure and functioning. Measurement endpoints include a) mortality to aquatic receptors, *Daphnia magna*, *Ceriodaphnia dubia*, and *Pimephales promelas*, b) mortality and growth of a common agricultural plant, *Lactuca sativa*, and c) mortality to a common soil invertebrate, *Eisenia foetida*, the redworm. Protocols for each of the bioassays can be found in Attachment 1.

### **Toxicity Evaluation**

This toxicity evaluation focuses on the toxicity of the perchlorate ion, rather than ammonium perchlorate. The rationale for this is the ammonium ion will act independently of the perchlorate ion and the ammonium ion will be degraded in the environment, effectively being replaced by a different cation (e.g. sodium or potassium), whereas the perchlorate ion is conserved within the environment. This effort does not attempt to specifically address potential effects of thyroid inhibition by perchlorate on populations of ecological receptors. Results of bioassays performed as part of this effort can be found in Table 1.

Based upon the toxicity tests conducted on *D. magna*, *C. dubia*, and *P. promelas* it is concluded

Table 1--Summary of Test Results

Test Species	Test Type	Endpoints (as mg/L ClO <sub>4</sub> )				
		LC <sub>50</sub>	NOEC	LOEC	ChV	IC25
<i>Daphnia magna</i>	Acute definitive	490				
<i>Pimephales promelas</i>	Acute definitive	1655				
<i>Eisenia foetida</i>	Acute definitive	4450/4450 <sup>1</sup>				
<i>Ceriodaphnia dubia</i>	Chronic definitive	66	10	33	18.2	17
<i>Pimephales promelas</i>	Chronic definitive	614	155	280	208	212
<i>Lactuca sativa</i>	Chronic definitive (soil)		<80	80	<80	78
<i>Lactuca sativa</i>	Chronic definitive (sand)		<80	80	<80	41
<i>Lactuca sativa</i>	Chronic definitive (soil) <sup>2</sup>		40	80	56.6	30
<i>Lactuca sativa</i>	Chronic definitive (sand) <sup>2</sup>		20	40	28.3	34.3

<sup>1</sup> 7-day/14 day

<sup>2</sup> Second set of lettuce growth tests initiated since NOEC was not identified in initial tests.

that perchlorate can cause mortality at relatively high exposure levels. The LC<sub>50</sub>s (48 hour tests for *D. magna* and *C. dubia*, and 96 hour test for *P. promelas*) are 490 mg/L, 66 mg/L and 1655 mg/L and 614 mg/L, respectively. The discrepancy between the two 96 hour test results for *P. promelas* appears to be due to age differences of the organisms used during the test. Therefore, the conservative interpretation of the results is to utilize the lower value, 614 mg/L 96hr LC<sub>50</sub>.

The results of the chronic toxicity tests conducted on *C. dubia* and *P. promelas* suggest that the safe concentration of perchlorate in water for these test species is about 18.2 mg/L and 208 mg/L, respectively, based upon the chronic values (ChV). Evaluation of the chronic toxicity utilizing the IC<sub>25</sub> is similar to the ChV calculations, being 17 mg/L and 212 mg/L, respectively for *C. dubia* and *P. promelas*.

The results of both the acute tests and the chronic tests indicate that fish (as represented by laboratory reared *P. promelas*) appear to be less sensitive to perchlorate than daphnids. However, it should be noted that the chronic endpoint for *C. dubia* includes reproduction.

Collectively, the water column toxicity tests suggest that perchlorate does not have a high degree of acute toxicity to water column organisms, such as fish and zooplankton. The results do suggest that impairment of reproduction and/or organism development are more sensitive endpoints for perchlorate toxicity. It should be noted that both of these endpoints are consistent with the known mechanism of perchlorate toxicity, competitive inhibition of iodide metabolism. For both the acute and chronic water tests the results suggest high levels of perchlorate are necessary in the exposure media (ppm exposure levels) to elicit adverse effects for these receptors under laboratory conditions.

Perchlorate has been demonstrated to adversely effect plants at high application rates, however the mechanism of the effect of perchlorate on plants is not known. It is also known that perchlorate that is found in fertilizers with apparently no adverse effect on the plants. The results of the first round of toxicity tests conducted on *L. sativa* suggested that the no effect concentration of perchlorate in soil was less than 80 mg/kg with the IC<sub>25</sub> of 41mg/kg. Since a NOEC was not identified, a second set of lettuce (*Lactuca sativa*) growth tests (using both soil and sand as the growth media) was initiated on 2 October 1998 with perchlorate concentrations ranging lower than in the initial (18 September 1998) tests. The soil test and sand were conducted with a concentration series of control, sodium control, 10, 20, 40, 80, and 160 mg/kg ClO<sub>4</sub>. The reported test endpoints are: percent germination, 28-day percent survival, and mean dry weight at the end of 28 days. In the soil test, germination was not significantly affected at any test concentration, and 28-day percent survival was not affected at perchlorate concentrations ranging from 10 through 80 mg/kg ClO<sub>4</sub>. Survival at 160 mg/kg ClO<sub>4</sub> was 36 percent and was significantly different from the control. Mean dry weight at the end of 28 days was lower in the 40 mg/kg ClO<sub>4</sub> (0.238 g/plant) than in the control (0.382 g/plant), but was not statistically different at P=0.05. Mean dry weight was statistically different than the control in the 80 mg/kg ClO<sub>4</sub> concentration. The resulting NOEC, LOEC, and ChV for the soil test were 40, 80, and 56.6 mg/kg ClO<sub>4</sub>, respectively. The IC<sub>25</sub> for the soil test was 30.0 mg/kg ClO<sub>4</sub> with a 95 percent



confidence interval of 5.0-64.4 mg/kg  $\text{ClO}_4$ .

The sand test results suggested that percent germination was a sensitive endpoint, and that four of the five tests concentrations resulted in significantly lower germination than the control. However, based on germination results in the first set of lettuce growth tests with higher perchlorate concentrations, it is felt that the significance of the germination results is anomalous due to higher control germination. Statistical analysis of the survival and growth data was performed, even though statistically the germination was affected. Analysis of the 28-day survival data detected a significant reduction at 80 and 160 mg/kg  $\text{ClO}_4$ . The mean dry weight data analysis detected a significant effect on plant growth at 40 mg/kg  $\text{ClO}_4$ . Thus, the 28-day NOEC, LOEC, and ChV were 20, 40, and 28.3 mg/kg  $\text{ClO}_4$ , respectively, based on dry weight as the most sensitive test endpoint. The IC25 for the sand test (34.3 mg/kg  $\text{ClO}_4$ ) was very similar to the soil test. It is not known why plant growth in the sand test was much lower in the 2 October 1998 test than in the 18 September 1998 test. The two IC25 values obtained from the toxicity tests are 41 mg/kg and 34.3 mg/kg. Information was not available on the effect of NaCl, for comparative purposes, and tests which have shown toxicity (e.g. leaf malformation) did not measure the actual soil concentration, which would allow comparisons with the tests conducted as part of this effort.

With respect to soil invertebrates, an  $\text{LC}_{50}$  was determined for earthworms (*E. foetida*). Both the 7 day and 14 day  $\text{LC}_{50}$ s were 4450 mg/kg. This suggests that for earthworms there is a threshold below which perchlorate has no lethal effects.

#### *Exposure Analysis*

Since perchlorate is not applied as a pesticide or have other designated general uses, and the array of potential ecosystems which may receive perchlorate exposure due to historical releases is large, it is difficult to develop specific assessment endpoints which will focus the ERA. This ERA, therefore, focuses on potential exposure pathways to ecosystems that represent known areas of perchlorate contamination and anticipated exposure pathways. It is appropriate to use exposure pathways as a means of refining the potentially appropriate assessment endpoints. The assessment endpoints would be those associated with surface waters, which receive groundwater discharges contaminated with perchlorate, sediments, associated with the groundwater discharge areas of contaminated groundwater, and soils, from which contaminated groundwater is discharging or to which contaminated water is applied.

There is currently no information to suggest that perchlorate is a bioconcentrating compound. That is, there is no information to suggest that perchlorate is actively concentrated in the tissues of higher organisms, either plants or animals. Information on human oral exposure suggests that the biological retention time for perchlorate is on the order of 6 hours. Based upon this, and the fact that most organisms maintain an osmotic balance in their bodies, it is unlikely that perchlorate will be actively retained by organisms. It therefore follows that a constant exposure to perchlorate would be necessary for adverse effects, or at least constant exposure during the critical development of the organism.

Although there is currently no information on the accumulation of perchlorate by plants, it is plausible to assume that the accumulation of perchlorate by plants will be similar to the accumulation of iodide, based upon iodide's molecular size and weight. The contaminated plant to herbivore/omnivore exposure pathway could be important if a forage plant species actually is a specific concentrator of perchlorate (or iodide), and if a receptor forages exclusively on plants that are exposed to perchlorate during critical life stages. However, we are currently unaware of any iodide accumulating plant species. These pathways can not be evaluated further due to lack of information.

A similar food chain exposure pathway is through soil invertebrates which live in soils through which contaminated groundwater discharges or that receive water containing perchlorate. Areas through which contaminated groundwater discharges are likely to be either very localized and/or discharging wetlands where the invertebrates are more likely to be aquatic, not terrestrial. For soils, it is assumed that in order for concentration of perchlorate to occur the receiving terrestrial environment would have to be highly arid with limited water application, as applications of water would result in a "washing" of the perchlorate through the soil. This is based upon the solubility of the perchlorate and the assumption that soils will not bind (retain) perchlorate unless there is evaporative concentration. As with the plant food chain, these pathways can not be evaluated further as we do not currently have ingestion toxicity benchmarks for terrestrial wildlife.

Since the existing information suggests that perchlorate is not retained (adsorbed or bound) by sediments, the anticipated exposure pathway of aquatic/ benthic organisms is at groundwater discharge areas. In these areas, elevated water concentrations of perchlorate could exist, as surface water dilution would not have yet occurred. For example, brook trout utilize groundwater upwellings for depositing their eggs (referred to as redds). Organisms that engage in this type of behavior (actively seeking groundwater discharge areas) may be at risk in areas of perchlorate contamination. Other organisms may be exposed directly to perchlorate dissolved in the water column. For example, pelagic fishes may be exposed to perchlorate as a function of concentration and time on site. As with the other food chain exposure pathways, the data limitations inhibit the ability to further evaluate these risks as we do not have wildlife toxicity benchmarks.

Several observations can be made based upon the limited fate and transport information available for perchlorate. It would be anticipated that the food/dietary exposure to ecological receptors would not exceed the ingestion rate multiplied times the abiotic media concentration. This assumption is predicated on the estimated biological retention time of perchlorate in the receptor organisms and that there is no anticipated mechanism for perchlorate build up or bioconcentration in tissues.

It is likely that the assessment endpoints associated with groundwater discharge areas would receive substantial exposures to perchlorate in solution. Since perchlorate is conserved within the aquatic environment, aquatic receptors are likely to have sustained exposures due to their contact with contaminated water throughout the annual cycle. Food chain exposures that have the potential to result in ecological risk are likely associated with the primary trophic level

beyond the plant or animal directly exposed to the perchlorate contaminated media. That is, because of the low biological residence time the degree of perchlorate exposure will rapidly decrease in subsequent trophic levels and will be highest for the "first level" exposure organism. It is likely that sustained exposure would be necessary in order to elicit an adverse response to most lower trophic level aquatic organisms, and possibly terrestrial receptors as well.

### *Risk Characterization*

Overall, for aquatic as well as terrestrial assessment endpoints, perchlorate does not appear to pose a substantive risk to the adult organisms, due to the comparatively high exposures necessary to result in lethality type responses.

The results of the amphibian toxicity tests suggest that embryonic toxicity is a plausible mechanism of ecological risk to assessment endpoints with sustained or seasonal exposure during critical life history stages.

Since the plant accumulation to foraging organism receptor pathway can not be evaluated due to a lack of information, it can not be concluded that there is no ecological risk to ecological receptors by this exposure pathway.

Based upon a lack of information of ingestion pathway toxicity benchmarks, the terrestrial food chain threats can not be fully evaluated. Therefore, we can not conclude that there are no terrestrial food chain ecological risks.

Organisms which utilize groundwater discharge areas may be at risk as they will be exposed to elevated perchlorate levels and may use the areas for nursery functions. Potential receptors include adult and juvenile fish, amphibians, and reptiles, larval and adult sediment invertebrates, and aquatic plants.

Plant communities which receive high application rates of perchlorate, or in which applied perchlorate is concentrated due to evaporative loss, may be at risk.

### **Uncertainties**

It has not been documented that perchlorate does not concentrate in the tissues of plants or animals and dietary absorption efficiencies are unknown

Wildlife toxicity exposure benchmarks do not currently exist. Some inferences may be made from the human health RfD once this value has been established. Data from experiments with mammals will also be useful.

The existing information suggests that the mechanism of ecological toxicity of perchlorate is related to competitive inhibition with iodide and, at extremely high exposures, osmotic stress. However, these mechanisms have not been firmly established and it cannot be stated whether

other mechanisms of toxicity exist.

## **Summary**

In this initial evaluation of the toxicity of perchlorate to ecological receptors, toxicity values were determined for several species thought to be representative of major groups of potential receptors in the field. Freshwater and soil inhabitants were evaluated. Terrestrial invertebrates, benthic organisms, or marine receptors were not addressed in this phase of the evaluation. The results of the toxicity tests conducted as part of this effort, suggest that perchlorate has a low degree of acute toxicity for these test organisms under standard laboratory conditions. The chronic toxicity tests conducted do suggest that developmental toxicity may occur at relatively high exposure concentrations, this finding being consistent with the known mechanism of perchlorate toxicity.

## **Recommendations for Further Studies**

Refinement of our understanding of exposure mechanisms of perchlorate is needed. Future studies on the toxicity and bioaccumulation potential of perchlorate are recommended to address the following concerns:

1. What is the potential for perchlorate accumulation in plants? What is the biological half-life?
2. What are the effects of chronic exposure of perchlorate to soil invertebrates?
3. Are there effects of perchlorate on plankton organisms that could impact consumers at higher trophic levels?
4. Is dietary exposure a significant pathway, through the evaluation of the accumulation and retention of perchlorate in plants and/or animals?

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### 3. RESULTS AND DISCUSSION - ADDENDUM

A second set of lettuce (*Lactuca sativa*) growth tests (soil and sand) with sodium perchlorate were initiated on 2 October 1998 with perchlorate concentrations lower than in the initial (18 September 1998) tests. The soil test (TN-98-490) and sand test (TN-98-491) were conducted with a concentration series of control, sodium control, 10, 20, 40, 80, and 160 mg/kg ClO<sub>4</sub>. The environmental parameters measured during the 28-day growth test are presented in Table 11, and the results of the tests are reported in Table 12. The reported test endpoints are: percent germination, 28-day percent survival, and mean dry weight at the end of 28 days.

In the soil test, germination was not significantly affected at any test concentration, and 28-day percent survival was not affected at 10 through 80 mg/kg ClO<sub>4</sub>. Survival at 160 mg/kg ClO<sub>4</sub> was 36 percent and was significantly different from the control. Mean dry weight at the end of 28 days was lower in the 40 mg/kg ClO<sub>4</sub> (0.238 g/plant) than in the control (0.382 g/plant), but was not statistically different at P=0.05. Mean dry weight was statistically different than the control in the 80 mg/kg ClO<sub>4</sub> concentration. The resulting NOEC, LOEC, and ChV for the soil test were 40, 80, and 56.6 mg/kg ClO<sub>4</sub>, respectively. The IC25 for the soil test was 30.0 mg/kg ClO<sub>4</sub> with a 95 percent confidence interval of 5.0-64.4 mg/kg ClO<sub>4</sub>.

The sand test results (Table 12) suggested that percent germination was a sensitive endpoint, and that four of the five tests concentrations resulted in significantly lower germination than the control. However, based on germination results in the first set of lettuce growth tests with higher perchlorate concentrations, it is felt that the significance of the germination results is anomalous due to higher control germination. Statistical analysis of the survival and growth data was performed, even though statistically the germination was affected. Analysis of the 28-day survival data detected a significant reduction at 80 and 160 mg/kg ClO<sub>4</sub>. The mean dry weight data analysis detected a significant effect on plant growth at 40 mg/kg ClO<sub>4</sub>. Thus, the 28-day NOEC, LOEC, and ChV were 20, 40, and 28.3 mg/kg ClO<sub>4</sub>, respectively, based on dry weight as the

most sensitive test endpoint. The IC25 for the sand test (34.3 mg/kg ClO<sub>4</sub>) was very similar to the soil test. It is not known why plant growth in the sand test was much lower in the 2 October 1998 test than in the 18 September 1998 test.



TABLE 1 SUMMARY OF WATER QUALITY RANGES MEASURED DURING TOXICITY TESTING FOR ARMSTRONG LABORATORY

<u>EA Test Number</u>	<u>Test Species</u>	<u>Test Type</u>	<u>Temperature (°C)</u>	<u>pH</u>	<u>Dissolved Oxygen (mg/L)</u>	<u>Conductivity (μS/cm)</u>
TN-98-424	<i>D. magna</i>	Acute Rangefinding	18.9-19.7 <sup>(a)</sup>	7.6-8.0	8.7-9.1	184-4900
TN-98-472	<i>D. magna</i>	Acute Definitive	19.6-20.9	7.5-8.1	7.8-9.1	182-1909
TN-98-425	<i>P. promelas</i>	Acute Rangefinding	23.6-25.5 <sup>(b)</sup>	7.4-8.1	7.6-8.1	184-5190
TN-98-474	<i>P. promelas</i>	Acute Definitive	24.1-25.4	7.7-8.3	7.2-7.9	171-3020
TN-98-423	<i>P. promelas</i>	Chronic Rangefinding	24.0-25.7	7.2-8.2	5.1-8.1	174-5050
TN-98-481	<i>P. promelas</i>	Chronic Definitive	24.0-25.5	7.4-8.2	5.6-8.4	168-1240
TN-98-480	<i>C. dubia</i>	Chronic Rangefinding	24.0-25.8	7.7-8.2	7.2-8.4	174-1431
TN-98-512	<i>C. dubia</i>	Chronic Definitive	24.0-25.4	7.0-8.2	7.5-8.4	306-463

(a) Temperature of test solution in one replicate was below the target range of  $20 \pm 1^\circ\text{C}$  at 24 hours.

(b) Temperatures of some test solutions were below the target range of  $25 \pm 1^\circ\text{C}$ .

TABLE 2 RESULTS OF *Daphnia magna* ACUTE TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Daphnia magna</i> (water flea)	<i>Daphnia magna</i> (water flea)
Test Type:	48-Hour Acute Rangefinding	48-Hour Acute Definitive
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )	Sodium perchlorate (NaClO <sub>4</sub> )
Dilution Water:	20% Dilute Mineral Water	20% Dilute Mineral Water
EA Test Number:	TN-98-424	TN-98-472
Test Initiation:	1610, 8 September 1998	1350, 24 September 1998
Test Termination:	1600, 10 September 1998	1420, 26 September 1998

Nominal Test Concentration (mg/L ClO <sub>4</sub> )	48-Hour Percent Survival
---	-----------------------------

Control	100
1	100
10	100
100	95
1000	35
5000	0

Na Control (1155 mg/L Na)	100
------------------------------	-----

Nominal Test Concentration (mg/L ClO <sub>4</sub> )	48-Hour Percent Survival
---	-----------------------------

Control	100
155	100
280	80
490	55
870	15
1550	0

Na Control (358 mg/L Na)	100
-----------------------------	-----

48-Hour LC50 (mg/L ClO<sub>4</sub>): 601 (340-973)<sup>(a)</sup>

490 (406-591)<sup>(a)</sup>

(a) Values in parenthesis represent 95 percent confidence limits.

TABLE 3 RESULTS OF *Pimephales promelas* ACUTE TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Pimephales promelas</i> (fathead minnow)	<i>Pimephales promelas</i> (fathead minnow)
Test Type:	96-Hour Acute Rangefinding	96-Hour Acute Definitive
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )	Sodium perchlorate (NaClO <sub>4</sub> )
Dilution Water:	20% Dilute Mineral Water	20% Dilute Mineral Water
EA Test Number:	TN-98-425	TN-98-474
Test Initiation:	1650, 8 September 1998	1215, 24 September 1998
Test Termination:	1635, 12 September 1998	1400, 28 September 1998

Nominal Test Concentration (mg/L ClO <sub>4</sub> )	96-Hour Percent Survival
---	-----------------------------

Control	100
1	100
10	100
100	100
1000	65
5000	0

Na Control (1155 mg/L Na)	95
------------------------------	----

Nominal Test Concentration (mg/L ClO <sub>4</sub> )	96-Hour Percent Survival
---	-----------------------------

Control	100
420	100
650	100
1000	100
1550	65
2400	0

Na Control (554 mg/L Na)	100
-----------------------------	-----

48-Hour LC50 (mg/L ClO<sub>4</sub>): 1128 (743-1712)<sup>(a)</sup>

1655 (1507-1817)<sup>(a)</sup>

(a) Values in parenthesis represent 95 percent confidence limits.

TABLE 4      SUMMARY OF ENVIRONMENTAL PARAMETERS MEASURED DURING *Eisenia foetida* TOXICITY TESTING

<u>EA Test Number</u>	<u>Test Type</u>	<u>Temperature (°C)</u>	<u>pH</u>	<u>Moisture (%)</u>
TN-98-489	Acute Rangefinding	25.0-26.0	5.9-6.2	21.8-27.2
TN-98-506	Acute Definitive	25.0-25.9	5.2-6.3	28.0-31.5

TABLE 5 RESULTS OF *Eisenia foetida* ACUTE TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Eisenia foetida</i> (red worm)	<i>Eisenia foetida</i> (red worm)
Test Type:	Acute Rangefinding	Acute Definitive
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )	Sodium perchlorate (NaClO <sub>4</sub> )
Dilution Media:	Artificial Soil	Artificial Soil
EA Test Number:	TN-98-489	TN-98-506
Test Initiation:	1720, 2 October 1998	1715, 9 October 1998
Test Termination:	1415, 16 October 1998	1615, 23 October 1998

Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	7-Day Percent Survival	14-Day Percent Survival	Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	7-Day Percent Survival	14-Day Percent Survival
Control	100	100	Control	100	100
1875	100	100	1000	100	100
3750	83	83	1800	100	100
7500	0	0	3200	100	100
15000	0	0	5600	10	10
30000	0	0	8700	0	0
Na Control (3465 mg/kg Na)	0	0	Na Control (2009 mg/kg Na)	97	93
LC50 (mg/kg ClO <sub>4</sub> ):	4725 (4299-5192) <sup>(a)</sup>	4725 (4299-5192) <sup>(a)</sup>		4450 (4213-4701) <sup>(a)</sup>	4450 (4213-4701) <sup>(a)</sup>

(a) Values in parenthesis represent 95 percent confidence limits.

TABLE 6 RESULTS OF *Ceriodaphnia dubia* CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Ceriodaphnia dubia</i> (water flea)			<i>Ceriodaphnia dubia</i> (water flea)			
Test Type:	Chronic Rangefinding			Chronic Definitive			
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )			Sodium perchlorate (NaClO <sub>4</sub> )			
Dilution Water:	20% Dilute Mineral Water			20% Dilute Mineral Water			
EA Test Number:	TN-98-480			TN-98-512			
Test Initiation:	1107, 29 September 1998			1650, 13 October 1998			
Test Termination:	1145, 7 October 1998			1607, 20 October 1998			
Nominal Test Concentration (mg/L ClO <sub>4</sub> )	48-Hour Percent Survival	8-Day Percent Survival	Mean Young Production (neonates/organism±SD)	Nominal Test Concentration (mg/L ClO <sub>4</sub> )	48-Hour Percent Survival	7-Day Percent Survival	Mean Young Production (neonates/organism±SD)
Control	100	80	18.9 (±13.3)	Control	100	80	22.8 (±7.8)
0.1	100	70	14.5 (±10.2)	1	100	80	22.2 (±5.9)
1	100	80	15.3 (±14.6)	3	100	90	26.3 (±7.9)
10	100	60	9.6 (±10.7)	10	100	70	22.2 (±9.0)
100	70	0 <sup>(b)</sup>	0 <sup>(b)</sup>	33	80	30 <sup>(a)</sup>	7.9 (±6.5) <sup>(b)</sup>
1000	10	0 <sup>(b)</sup>	0 <sup>(b)</sup>	100	30	10 <sup>(a)</sup>	0 <sup>(b)</sup>
Na Control (231 mg/L Na)	100	70	15.2 (±15.2)	Na Control (23.1 mg/L Na)	100	70	25.7 (±9.3)
<u>Endpoints (as mg/L ClO<sub>4</sub>)</u>							
48-Hour LC50:	202 (79-534) <sup>(c)</sup>			66 (40-144) <sup>(c)</sup>			
NOEC:	10			10			
LOEC:	100			33			
ChV:	31.6			18.2			
IC25:	2.2 (0.1-17.1) <sup>(c)</sup>			17.0 (8.1-20.5) <sup>(c)</sup>			

(a) Concentration was significantly different from the control based on percent survival.

(b) Concentrations which have no surviving organisms or significant mortality are not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

TABLE 7 RESULTS OF *Pimephales promelas* CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Pimephales promelas</i> (fathead minnow)			<i>Pimephales promelas</i> (fathead minnow)			
Test Type:	Chronic Rangefinding			Chronic Definitive			
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )			Sodium perchlorate (NaClO <sub>4</sub> )			
Dilution Water:	20% Dilute Mineral Water			20% Dilute Mineral Water			
EA Test Number:	TN-98-423			TN-98-481			
Test Initiation:	1655, 8 September 1998			1447, 29 September 1998			
Test Termination:	1610, 15 September 1998			1700, 6 October 1998			
Nominal Test Concentration (mg/L ClO <sub>4</sub> )	96-Hour Percent Survival	7-Day Percent Survival	Mean Biomass (mg/organism±SD)	Nominal Test Concentration (mg/L ClO <sub>4</sub> )	96-Hour Percent Survival	7-Day Percent Survival	Mean Biomass (mg/organism±SD)
Control	98	98	0.838 (±0.075)	Control	100	100	0.687 (±0.036)
1	98	98	0.842 (±0.105)	87	100	100	0.648 (±0.055)
10	100	100	0.864 (±0.015)	155	100	100	0.646 (±0.072)
100	100	98	0.777 (±0.076)	280	98	68 <sup>(a)</sup>	0.359 (±0.038) <sup>(b)</sup>
1000	15	0 <sup>(b)</sup>	--	490	60	10 <sup>(a)</sup>	0.033 (±0.045) <sup>(b)</sup>
2000	0	0 <sup>(b)</sup>	--	870	28	0 <sup>(b)</sup>	--
Na Control (462 mg/L Na)	65	23 <sup>(a)</sup>	0.049 (±0.024) <sup>(b)</sup>	Na Control (201 mg/L Na)	73	13 <sup>(a)</sup>	0.037 (±0.032) <sup>(b)</sup>
<u>Endpoints (as mg/L ClO<sub>4</sub>)</u>							
96-Hour LC50:	397 (335-471) <sup>(c)</sup>			614 (540-714) <sup>(c)</sup>			
NOEC:	100			155			
LOEC:	1000			280			
ChV:	316			208			
IC25:	264 (139-362) <sup>(c)</sup>			212 (175-231) <sup>(c)</sup>			

(a) Concentration was significantly different from the control based on percent survival.

(b) Concentrations which have no surviving organisms or significant mortality are not included in statistical analyses.

(c) Values in parenthesis represent 95 percent confidence limits.

TABLE 8      SUMMARY OF ENVIRONMENTAL PARAMETERS MEASURED DURING *Lactuca sativa* TOXICITY TESTING

<u>EA Test Number</u>	<u>Test Type</u>	<u>Temperature (°C)</u>	<u>Light (lux)</u>	<u>Relative Humidity (%)</u>
TN-98-447	Chronic Definitive (soil)	24.8-28.5	4410-7850	40.0-52.0
TN-98-448	Chronic Definitive (sand)	24.8-28.5	5930-8400	40.0-52.0



TABLE 9 RESULTS OF *Lactuca sativa* CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Lactuca sativa</i> (buttercrunch lettuce)			<i>Lactuca sativa</i> (buttercrunch lettuce)			
Test Type:	Chronic Definitive			Chronic Definitive			
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )			Sodium perchlorate (NaClO <sub>4</sub> )			
Dilution Media:	Soil			Sand			
EA Test Number:	TN-98-447			TN-98-448			
Test Initiation:	1520, 18 September 1998			1530, 18 September 1998			
Test Termination:	1500, 16 October 1998			1541, 16 October 1998			
Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	% Germination	28-Day % Survival	28-Day Mean Dry Weight (g/plant±SD)	Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	% Germination	28-Day % Survival	28-Day Mean Dry Weight (g/plant±SD)
Control	77	100	0.111 (±0.005)	Control	77	100	0.183 (±0.012)
80	93	100	0.083 (±0.014) <sup>(a)</sup>	80	83	100	0.094 (±0.011) <sup>(a)</sup>
180	90	100	0.013 (±0.005) <sup>(a)</sup>	180	80	61	0.022 (±0.007) <sup>(a)</sup>
420	83	74	0.016 (±0.011) <sup>(a)</sup>	420	93	17 <sup>(b)</sup>	0.070 (±0.094) <sup>(c)</sup>
1000	57	0 <sup>(c)</sup>	--	1000	83	0 <sup>(c)</sup>	--
2400	3 <sup>(d)</sup>	0 <sup>(c)</sup>	--	2400	40 <sup>(d)</sup>	0 <sup>(c)</sup>	--
Na Control (554 mg/kg Na)	90	100	0.081 (±0.012) <sup>(a)</sup>	Na Control (554 mg/kg Na)	97	100	0.134 (±0.013)
<u>Chronic Endpoints (as mg/kg ClO<sub>4</sub>)</u>							
NOEC:	<80			<80			
LOEC:	80			80			
ChV:	<80			<80			
IC25:	78 (26-113) <sup>(e)</sup>			41 (31-54) <sup>(e)</sup>			

(a) Concentration was significantly different from the control based on mean dry weight.

(b) Concentration was significantly different from the control based on percent survival.

(c) Concentrations which have no surviving organisms or significant mortality are not included in statistical analyses.

(d) Concentration was significantly different from the control based on percent germination.

(e) Values in parenthesis represent 95 percent confidence limits.

TABLE 10 SUMMARY OF REFERENCE TOXICANT TESTING

<u>Test Species</u>	<u>Organism Lot Number</u>	<u>Reference Toxicant Test Number</u>	<u>Reference Toxicant</u>	<u>Endpoint</u>	<u>Acceptable Control Chart Limits</u>
<i>Daphnia magna</i>	NA	RT-98-171	Potassium chloride (KCl)	48-Hour LC50:	487 mg/L KCl 427-710 mg/L KCl
<i>Ceriodaphnia dubia</i>	NA	RT-98-201	Sodium chloride (NaCl)	48-Hour LC50:	1.7 g/L NaCl 1.1-3.0 g/L NaCl
<i>Pimephales promelas</i>	NA	RT-98-169	Potassium chloride (KCl)	48-Hour LC50:	1000 mg/L KCl 485-1063 mg/L KCl
<i>Eisenia foetida</i>	EF-008	RT-98-188	2-chloroacetamide (C <sub>2</sub> H <sub>4</sub> CINO)	48-Hour LC50:	146 mg/L C <sub>2</sub> H <sub>4</sub> CINO 141-419 mg/L C <sub>2</sub> H <sub>4</sub> CINO
	EF-009	RT-98-198	2-chloroacetamide (C <sub>2</sub> H <sub>4</sub> CINO)	48-Hour LC50:	122 mg/L C <sub>2</sub> H <sub>4</sub> CINO 98-423 mg/L C <sub>2</sub> H <sub>4</sub> CINO
<i>Lactuca sativa</i>	K-409	RT-98-215	Copper sulfate (CuSO <sub>4</sub> )	120-Hour IC50:	2.23 mg/L Cu 2.09-2.63 mg/L Cu

NA=Not applicable; reference toxicant tests performed monthly on in-house organism cultures.

ADDENDUM

TABLE 11 SUMMARY OF ENVIRONMENTAL PARAMETERS MEASURED DURING *Lactuca sativa* TOXICITY TESTING

<u>EA Test Number</u>	<u>Test Type</u>	<u>Temperature (°C)</u>	<u>Light (lux)</u>	<u>Relative Humidity (%)</u>
TN-98-490	Chronic Definitive (soil)	24.3-25.6	6360-7720	39.1-51.0
TN-98-491	Chronic Definitive (sand)	24.3-25.6	6360-8380	39.1-51.0

# ADDENDUM

TABLE 12 RESULTS OF *Lactuca sativa* CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	<i>Lactuca sativa</i> (buttercrunch lettuce)				<i>Lactuca sativa</i> (buttercrunch lettuce)			
Test Type:	Chronic Definitive				Chronic Definitive			
Test Material:	Sodium perchlorate (NaClO <sub>4</sub> )				Sodium perchlorate (NaClO <sub>4</sub> )			
Dilution Media:	Soil				Sand			
EA Test Number:	TN-98-490				TN-98-491			
Test Initiation:	1500, 2 October 1998				1500, 2 October 1998			
Test Termination:	1400, 30 October 1998				1520, 30 October 1998			
Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	% Germination	28-Day % Survival	28-Day Mean Dry Weight (g/plant±SD)	Nominal Test Concentration (mg/kg ClO <sub>4</sub> )	% Germination	28-Day % Survival	28-Day Mean Dry Weight (g/plant±SD)	
Control	80	100	0.382 (±0.076)	Control	93	97	0.073 (±0.007)	
10	60	100	0.398 (±0.106)	10	67 <sup>(a)</sup>	100	0.062 (±0.007)	
20	77	97	0.348 (±0.067)	20	73	100	0.061 (±0.002)	
40	80	100	0.238 (±0.104)	40	70 <sup>(a)</sup>	100	0.052 (±0.010) <sup>(b)</sup>	
80	77	94	0.118 (±0.050) <sup>(b)</sup>	80	63 <sup>(a)</sup>	63 <sup>(c)</sup>	0.014 (±0.006) <sup>(d)</sup>	
160	83	36 <sup>(c)</sup>	0.021 (±0.009) <sup>(d)</sup>	160	53 <sup>(a)</sup>	5 <sup>(c)</sup>	0.004 <sup>(d)</sup>	
Na Control	43	100	0.203 (±0.152)	Na Control	83	100	0.069 (±0.008)	

## Chronic Endpoints (as mg/kg ClO<sub>4</sub>)

NOEC:	40	20
LOEC:	80	40
ChV:	56.6	28.3
IC25:	30.0 (5.0-64.4) <sup>(e)</sup>	34.3 (9.1-58.7) <sup>(e)</sup>

- (a) Concentration was significantly different from the control based on percent germination; however, statistical significance was considered anomalous (see text).  
 (b) Concentration was significantly different from the control based on mean dry weight.  
 (c) Concentration was significantly different from the control based on percent survival.  
 (d) Concentrations which have significantly less survival than the control are not included in statistical analyses for dry weight.  
 (e) Values in parentheses represent 95 percent confidence limits.